Embryonic Stem Cell-Derived T Lymphocyte Precursors

It has become increasingly clear that the process of thymic education involves complex interactions between developing T lymphocytes and various stromal components of the thymus. Most studies aimed at addressing these issues have been hampered by the inaccessibility of significant numbers of primitive "uneducated" T cell precursors. The goal of this work is the full characterization of a novel *in vitro* developmental system that promotes the generation of primitive T cell precursors from undifferentiated embryonic stem (ES) cells in culture. The advantages of this approach are several. We will gain access to unlimited numbers of these cells, we can introduce genetic changes in the ES cells with relative ease and we will be able to analyze the affects of these changes on the differentiating progeny.

We have already established two models that allow the use of ES cells as a source of T lymphocyte precursors. In the first system, ES-derived cell lines were established *in vitro* after immortalization with oncogenic retroviruses. They gave rise to mature αβ- T lymphocytes after adoptive transfer *in vivo*. The transformation event had lead to malignant growths within the lymphoid system in some of the recipients. Therefore, we investigated a second approach by propagating ES-derived precursors in the absence of retroviruses. We could demonstrate that these cells repopulated fetal thymus lobes *in vitro*. However, the frequency of T lymphocyte precursors in these lines proved to be low. These studies, nevertheless, T lymphocyte precursors can be cultured without the need of viral transformation.

These systems will be optimized further with the following goals in mind:

I. Characterization of ES-Derived T Lymphocyte Precursors:

We have established culture systems that allow the differentiation of ES cells into T lymphocyte precursors. However, the frequency of pre-T cells in these ES-derived mixed populations is very low. Therefore, we will further characterize these mixed populations, to identify the T lymphocyte precursors, and to optimize culture conditions enriching for these precursors.

II. Defining Mechanisms Establishing Fetal and Adult Thymic Development:

Fetal and adult thymi show distinct differences in their developmental patterns. ES-derived lymphoid precursors might be representative of a developmental stage corresponding to fetal liver cells. However, it is presently not known whether all these differences are imprinted on the level of the stem cell or are caused by the thymic environment. Therefore, we will pursue this point because of two reasons: a) we will perform experiments that will help us to understand the mechanisms that determine thymic developmental pattern; b) in conjunction with these experiments, we will define whether thymi repopulated with ES-derived precursors resemble fetal or adult organs.